





# Natural Materials, Systems & Extremophiles

06 03 2012

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## 2012 AFOSR SPRING REVIEW



NAME: Dr Hugh C. DeLong

### **BRIEF DESCRIPTION OF PORTFOLIO:**

The goals of this program are to: 1) study, use, mimic, or alter how biological systems accomplish a desired (from our point of view) task, and 2) enable them to task-specifically produce natural materials and systems. Both goals are to advance or create future USAF technologies.

### **LIST SUB-AREAS IN PORTFOLIO:**

Biomimetics
Biomaterials
BioInterfacial Sciences
Extremophiles





## **Program Vision**



- This program not only wants to mimic existing natural systems, but also to create new capabilities in or with these organisms for more precise control over system production.
  - Protect Human Assets Finding and Defending against militarily significant threats to humans
  - Enhance Materials Performance Use natural systems to enhance or create new materials
  - Enhance System Operation Mimic nature's ability to find, track, and survive the enemy





# **Program Trends – Program Constant with Additions Coming From Outside Program**





Chromophores/Bioluminescence – Bio-X STT phase 1 focus. One of its discoveries are now used by AFRL TDs, Navy & several Univ Pl's



Bio-camouflage – FY09 PBD 709 program: iridiphores, leucophores, chromatophores, papillae, control system. Linked: FY11 AFRL/RX pgm



Structural Coloration – new area, several PIs moving in and out; MURI (Harvard)



Biopolymers – Mainly silk but looking at other biopolymers. The silk work is well integrated with AFRL; many exchanges of personnel & material. Some PIs moving out with biocomposites increasing.



Biomolecular assembly – New MURI (Northwestern), existing MURI (Georgia Tech), rest has remained constant.



Peptide Mediated Materials Synthesis – The efforts are focused on discovering the nature of the mechanism behind this.



Extremophile survival – Looking at mechanisms of protein activity under extreme conditions with the goal to transfer good ideas into weaker systems. Fewer PIs left that perform this type of work.



Biocombinatorics – New BRI looking at Bio based combinatorics from a bio-nano-info basis



# Other Organizations That Fund Related Work



- Chromophores I currently have two grants plus work in AFRL. The work of other organizations is almost exclusively on reporter technology. The interest of the AFOSR program is on wavelength, intensity, and lifetime as it pertains to marking items.
- Silk DARPA has contributed to my existing program. ARO has a single grantee. ONR funds a single investigator. NSF has several single PI grants.
- Structural Coloration/Bio-Camouflage ONR has a MURI focused on vision aspect. ARO has a single grant with ICB PI. NSF has just single PI grants.
- Biomolecular assembly A number of funding organizations are interested in this area, so the AFOSR program is focused on soft lithography, peptide binding, and self or directed assembly for materials. AFRL program works closely with this group for both relevance and guidance.
- Extremophiles NASA has funded this area and focused on the origins of life.
   The focus of the AFOSR program is on radiation protection mechanisms, biotemplating, and biopolymers that can exist in extreme environments. ARO is focused on spore formers.





## Sensory Mimics (Biomimetics)



- Study principles, processes, and designs as well as manipulate sensors/processing systems
- Mimicking of sensor denial systems
- The Future of Sensory Mimics:
  - Mimicking sensor motifs used by animal for flight operations
  - Complex autonomous materials (skin-like; sensing, regulating, healing) (w/ L. Lee)
  - Understand the complex nature of predator-prey avoidance to hide in plain sight
- AF Relevance: Sensitivity, Self-healing, Stealthy



# Study and Characterization of Bio-photonic Systems, P. Vukusic, U. Exeter



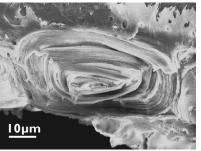


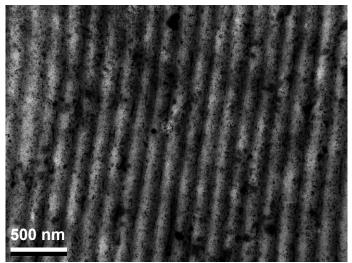
Interested in studying the relationship between the interplay of hierarchical structures on different length scales

#### The natural system

Concentric multilayers in the epidermal cells of a plant seed

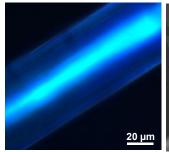


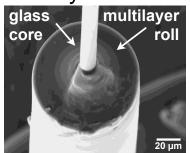


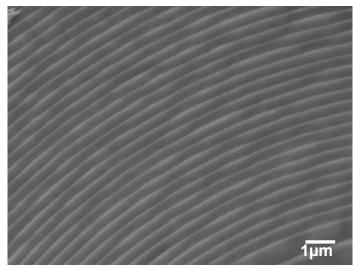


### The artificial counterpart

Artificial multilayer photonic fiber with 60 - 200 layers





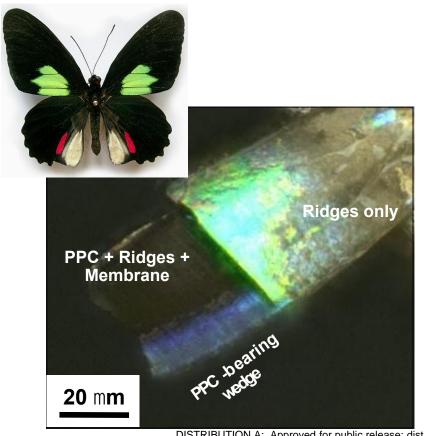


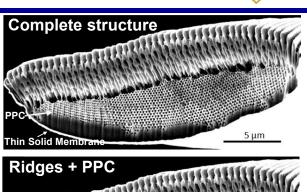


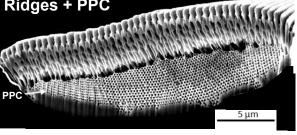
# Precise deconstruction of hierarchical biological structures

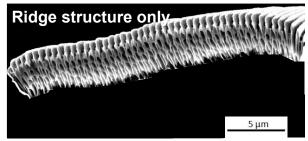


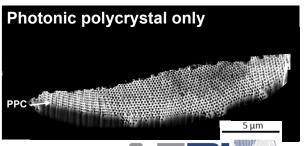
Deconstruction of a wing scale of the tropical butterfly Papilio palinurus to analyze the optical performance of the individual elements - ridges, a photonic polycrystal and the supporting membrane - and to understand their optical interplay













### 3D Model Physical Fabrication

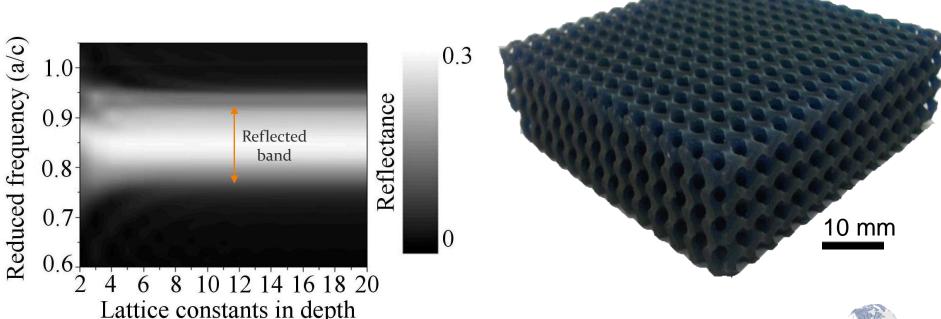


#### Maxwell's equations are scalable:

• Direct laser writing: resolution issues for **complex** structures therefore characterise in the microwave regime.

Stereolithography allows 3D printing of complex structures:

- Resolution ~50µm
- Uses dielectrics
- Potential for metals and stretchable materials





## Natural Materials (Biomaterials)



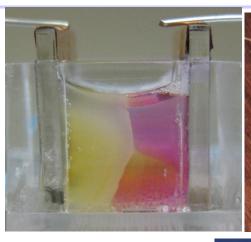
- Mimicking of natural materials or systems
- Using organisms as natural material factories for new materials
- Using existing natural materials/organisms as novel materials
- The Future of Natural Materials:
  - Natural Materials that can withstand extreme environments
  - New optical and electronic materials based on biology's capability to self-assemble
  - New materials grown to order by a biological organism (w/ J. Fuller)
  - Used as structural materials for advanced UAV systems
- AF Relevancy: Improved performance, Shape, Composites



### <u>Dynamic Silk Materials</u> – electrogelation and silk processing for new functional materials David Kaplan - Tufts University









Electrogelation – mechanisms & modes of materials formation and functions





**Objective:** To understand and exploit the novel dynamic properties of silks, including under applied electric fields and in aqueous environments, as a route to new functionalized materials.

### Approach:

- Mechanisms characterization of silk proteins under electric fields – structure, morphology, behavior
- *Protein assembly* device designs to study silk assembly under electric fields, adhesion
- *Materials characterization* use novel analytical tools to characterize assembly

#### **Progress:**

- New insight into mechanisms pH, morphology, improved model
- New high performance materials and properties generated from silk through the process
- New silk-electronic interfaces
- New dynamic silk-based materials

#### Impact:

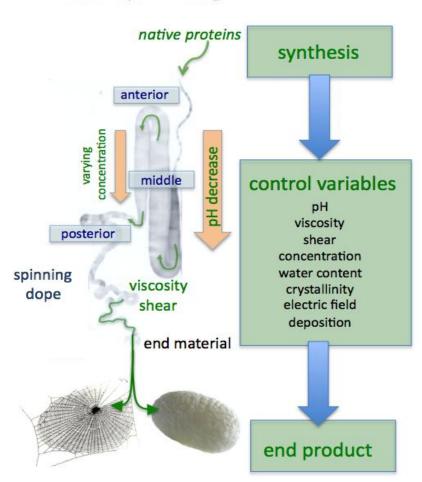
- High performance silk-based materials and processes – fibers, films, coatings
- New dynamic silk-based material systems
- New reversible adhesives
- New nano- and micro-composite materials





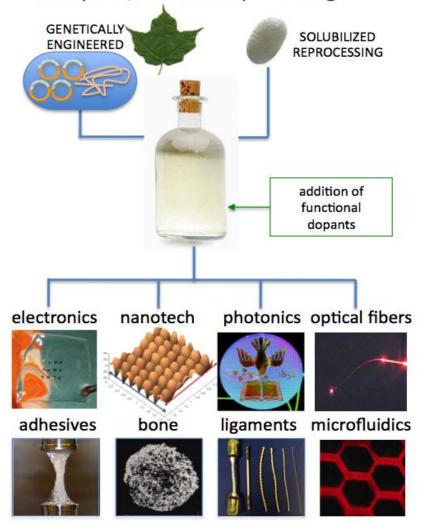
# Biomimetic Processing of Silk Protein → New Materials and Devices

#### natural processing



Theme – control of water content

#### bioinspired/biomimetic processing





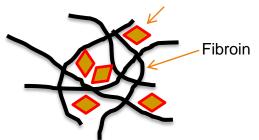
## **Enzyme Entrapment in Silk** Rajesh Naik, AFRL-RX





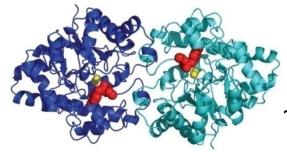
### Advantages of Enzyme Stabilization in Silk fibroin Films

Enzyme



- Large hydrophobic domains and small hydrophilic spacers
- Fibroin Crystalline domains (β-sheets) and less organized more flexible domains (more hydrated)
  - Microenvironments sufficient hydration
  - Controlled released based on silk processing conditions
  - Enzymes with varied molecular weights can be entrapped

#### Silk Fibroin



~ 45kDa mol. wt ■

**Organophosphate Hydrolase** (OPH)







**Nerve Agents** 



Silk Fibroin (OPH)

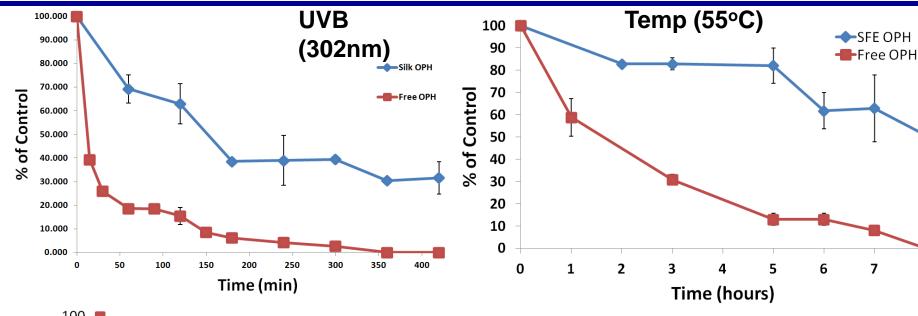
(In collaboration with David Kaplan)

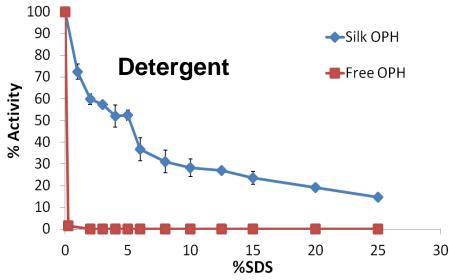




## Stability of OPH Entrapped in Silk







Increased protection against UV, heat and detergent of OPH-Silk films

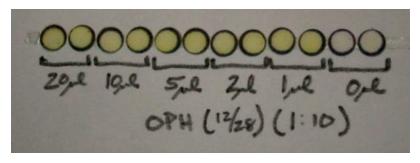




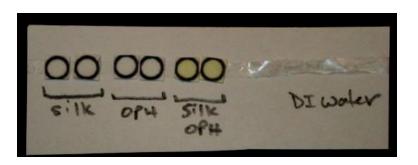
## Paper Based Microfluidics for Organophosphates



# Activity of OPH-Silk spotted onto paper



OPH is unstable on paper



LOD = 25 μM

year Inemails 10

10

10

5

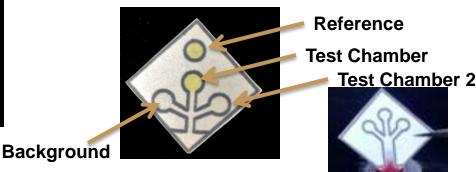
10

15

20

25

Concentration (mM)
Microfludic Paper Assay
for Chem agents (µPAC)



In Collaboration with Josh Hagen, AFRL/RH





# Natural/Synthetic Interfaces (Biointerfacial Sciences)

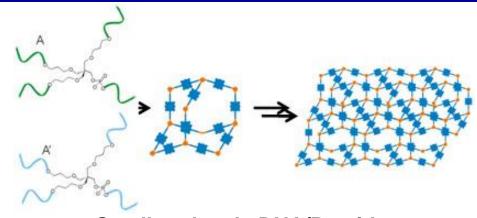


- Biotic-biotic or the biotic-abiotic interface.
- Bionanotechnology and biomesotechnology.
- Self-assembly, directed assembly
- The Future of Natural/Synthetic Interfaces:
  - biocatalysts for electrical power systems (providing low signature, long life ISR capability)
  - sensor applications in extreme environments
  - bio-optics and bio-electronics (w/ G. Pomrenke & H. Weinstock)
- AF Relevancy: Nanofabrication constraints on design & production

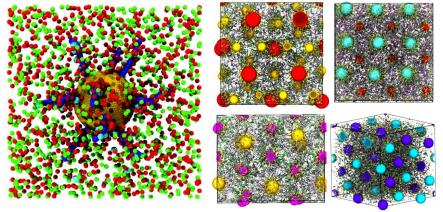


# Bio-Programmable 1-, 2-, & 3-D Materials, Chad A. Mirkin, Northwestern University

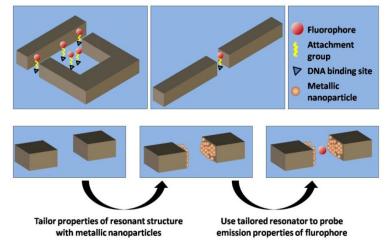




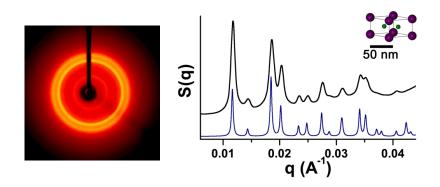
Small-molecule DNA/Peptide Hybrid Structures (Nguyen, Rosi, Mirkin)



Theoretical Examination of Nanoparticle
Assembly and Properties
(Schatz, Olvera, Rosi, Mirkin)



Large-Scale Patterned Metamaterial Arrays (Atwater, Schatz, Mirkin)



X-Ray Characterization of Materials (Bedzyk, Rosi, Mirkin)

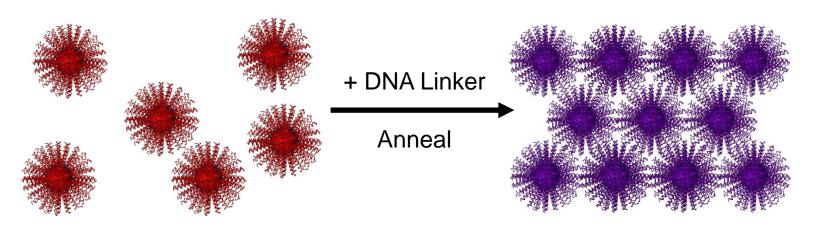
17



# Key Hypothesis of DNA-Programmed Assembly:



In the context of DNA-programmable nanoparticle assembly, the structures that represent thermodynamic minima rather than kinetics will maximize the number of nearest neighbors that can form DNA connections.



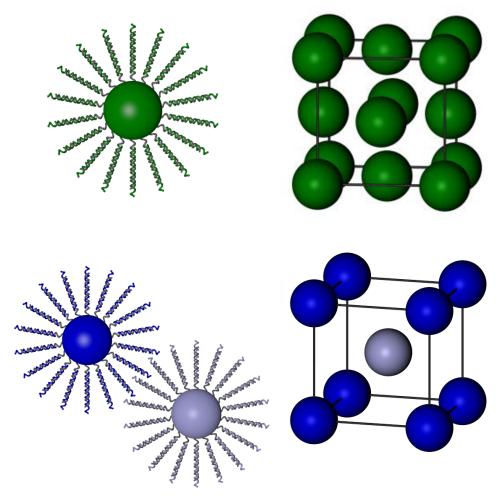
**Developed Five Rules of DNA-Programmed Assembly** 



# Rule #1: Particles of Equal Hydrodynamic Radius will Maximize Complementary Nearest Neighbors



#### **Face-Centered Cubic Lattice**



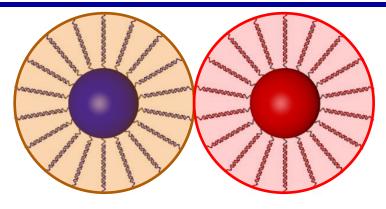
**Body-Centered Cubic Lattice** 

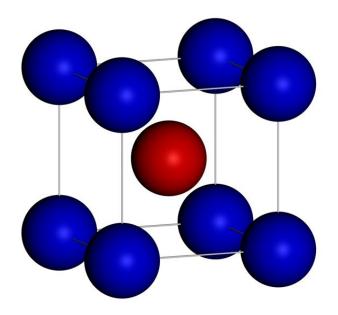




# Rule #2: The overall hydrodynamic radius of a DNA-NP dictates its assembly and packing behavior





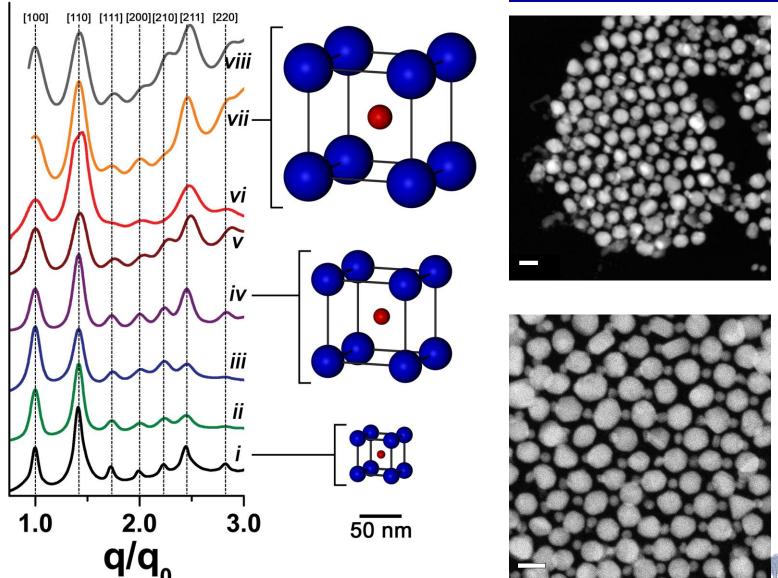


**Body-Centered Cubic Lattice** 



# Rule #2: The overall hydrodynamic radius of a DNA-NP dictates its assembly and packing behavior





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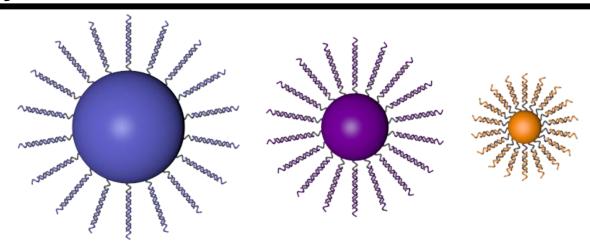
All Scale Bars = 50 nm



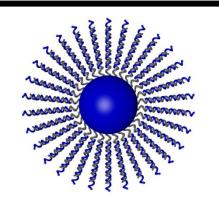
### Rule #3: Particle Hydrodynamic Size ratio and DNA Linker Ratio Dictate the Thermodynamically Favored Crystal Structure

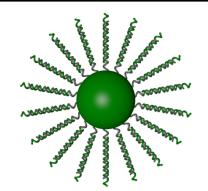


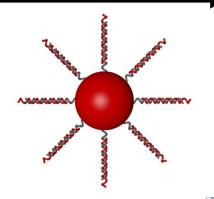
### **Hydrodynamic Radius**



### **DNA Linkers per NP**







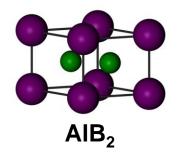


### Rule #3: Particle Hydrodynamic Size ratio and DNA Linker Ratio Dictate the Thermodynamically Favored Crystal Structure



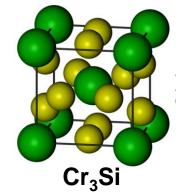
H<sub>d</sub> Size Ratio: **0.64** 

Linker Ratio: 2.4



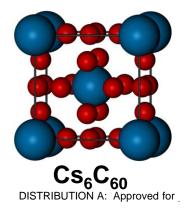
H<sub>d</sub> Size Ratio: **0.37** 

Linker Ratio: 2.0



H<sub>d</sub> Size Ratio: **0.35** 

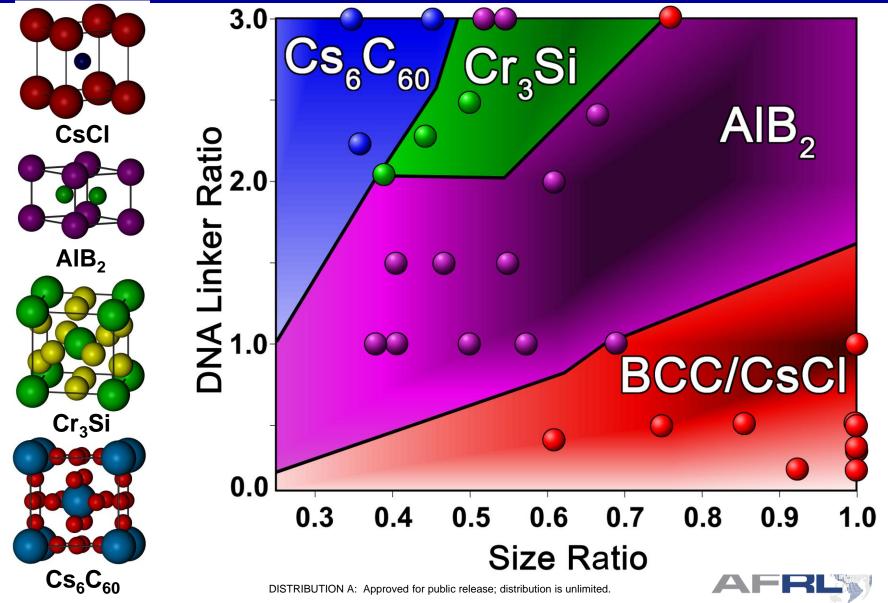
Linker Ratio: 3.0





Rule #4: Two Systems With the Same Hydrodynamic Size Ratio and DNA Linker Ratio Exhibit the Same Thermodynamic Product

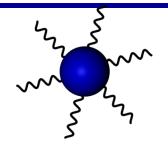






# Rule #5: The Most Stable Crystal Will Maximize All Possible Types of DNA SequenceSpecific Hybridization Interactions

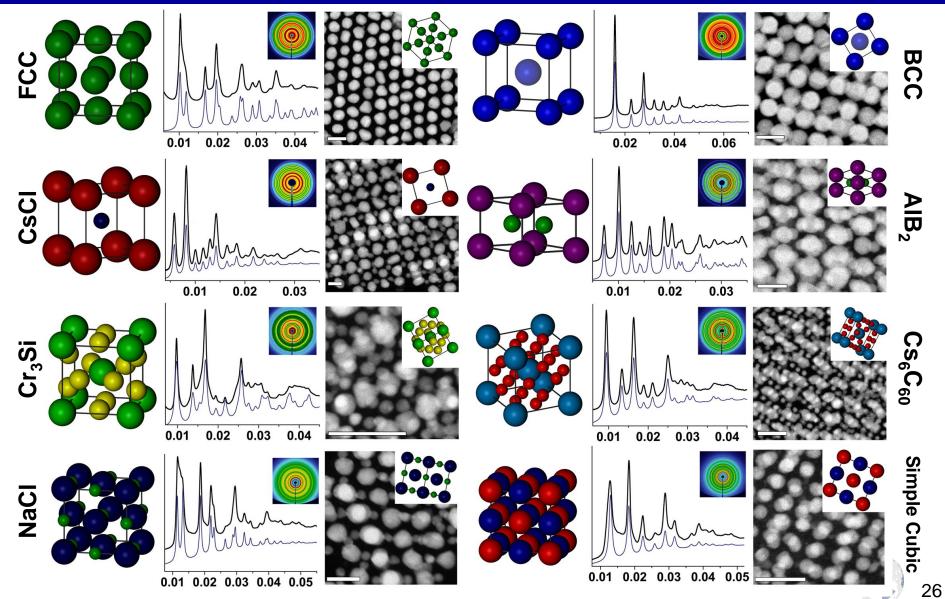






# DNA-Programmable Nanoparticle Materials by Design

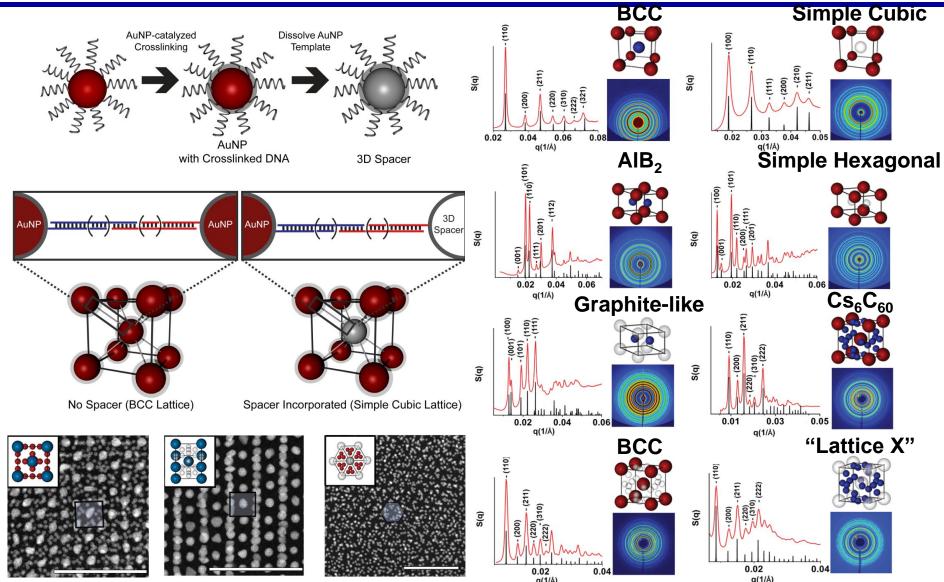






# Expanding Lattice Versatility with a 3-D "Hollow Spacer"



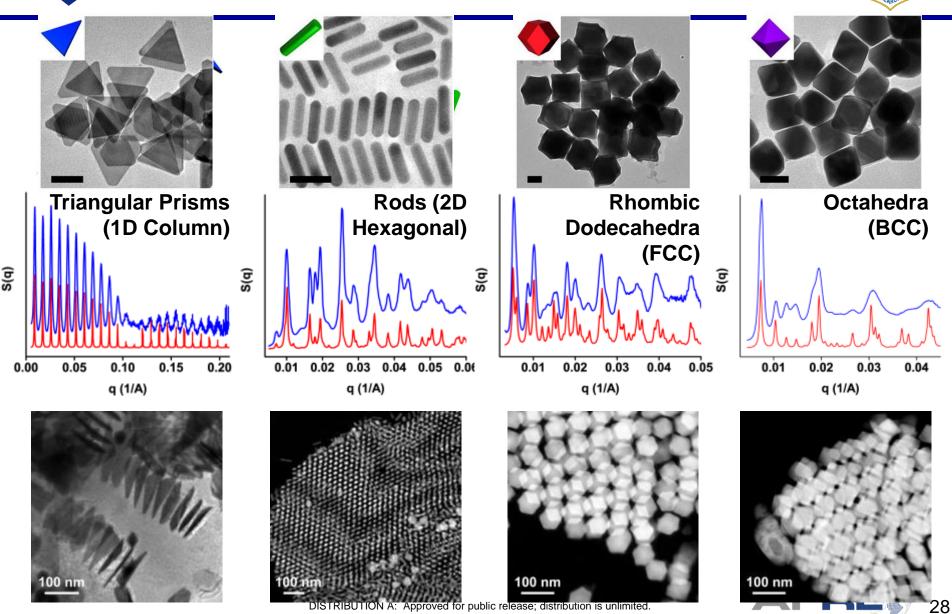


DISTRIBUTION A: Approved for public rollows, distribution is



### Nanoparticle Valency Imposed by Flat **Surfaces Yields Ordered Superlattices**







### Physical Mechanisms of Natural Systems Under Environmental Distress (Extremophilies)



- Focused on discovering and understanding basic natural mechanisms
- Increasingly used as catalysts, sensors, and as materials, so necessary to understand how can use in extreme environments, while incorporating change.
- The Future of Physical Mechanisms of Natural Systems Under Environmental Distress:
  - the mechanisms for survival and protein stability in extremophilic archaea & their viruses, and enzymatic engineering for faster catalysis in material degradation designs.
- AF Relevancy: New catalysts, sensors, and as materials

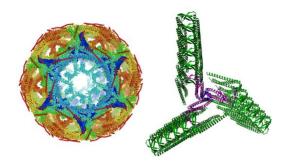


### **Engineering Ultrastable Protein Filaments into** 2D & 3D Templates for Materials Design-**Douglas Clark, UC Berkeley**

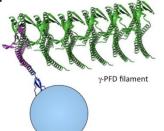


#### From Hyperstable Filaments to Self-Assembling Ovaloids: Expanding the Dimensions of Protein Design

Current protein templates and architectures for nanoscale device fabrication are limited to natural molecules owing to difficulties associated with generating new full-domain protein shapes.

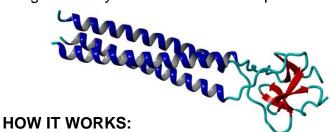


We have demonstrated that the y-PFD is exceptionally stable and can be engineered for numerous possible applications.



#### **MAIN ACHIEVEMENTS:**

- Charaterized y-PFD assembly as function of T; discovered new filament morphology.
- Designed and expressed 2D and 3D connector proteins and demonstrated binding with γ-PFD.
- Developed y-PFD variant with greater thermostability.
- Engineered system for secretion of y-PFD.



#### • 3D Protein connectors are based on hub region of the cage-like protein clathrin and the foldon from viral protein fibritin.

- Ultrastable y-PFD mutant rationally engineered using structure-stability relationships but filament formation of mutant must still be engineered
- y-PFD secreted from B. subtilis for assembly of filaments ex vivo.

#### **ASSUMPTIONS AND LIMITATIONS:**

2D structure of "pinwheel" construct requires further confirmation; assembly of more complex 3D structures not yet accomplished.

DISTRIBUTION A: Approved for public release; distribution is unlimited.

Generating highly stable proteins that assemble into 2D and 3D shapes of controllable size and symmetry will increase the dimensional space for template-based construction **IMPACT** of advanced biomaterials.



Rational design and construction of modular 2D and 3D protein architectures that will serve as lattices and scaffolds in protein-based and hybrid biomaterials.

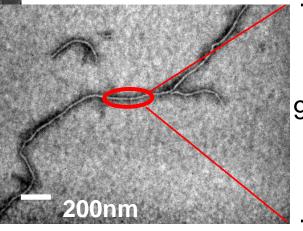


**END-OF-PHASE** GOAL

QUANTITATIVE

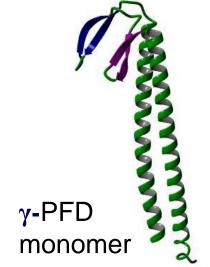
# Filamentous $\gamma$ -Prefoldin ( $\gamma$ -PFD)

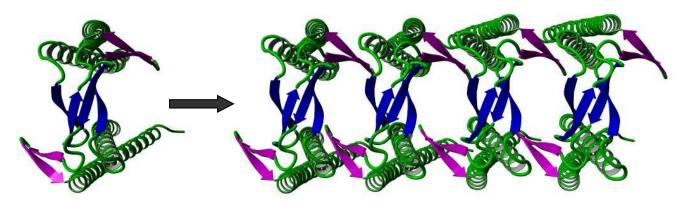
Methanocaldococcus jannaschii





*γ-prefoldin (γ-PFD)* 





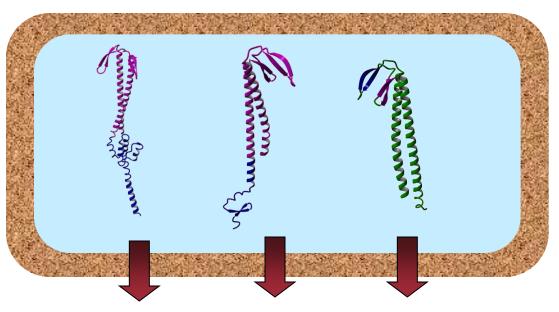
**γ-**PFD dimer

γ-PFD filament\_

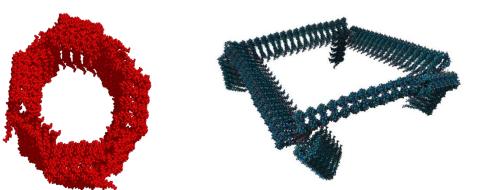
# Microbial factories for controlling protein assembly



B. subtilis engineered to express γ–PFD protein parts



Rods and connectors expressed and secreted from *B.* subtilis in a controlled manner



Controlled assembly of higherorder structures ex vivo





### **Transitions**



- AFRL/RX Collaboration with GE on bio-inspired photonic sensors (CRADA)
- AFRL/RX DTRA funding for biofunctionalized textiles for Chem-Bio (jointly with AFRL/RH)
- AFRL/RX Invention disclosure filed on Halamine functionalized biomaterials for decon application
- Northwestern Patent application "Tunable compliant optical metamaterial structures (US 13/200,273)
- Connecticut College Luciferase product development of patent (US Patent # 7,807,429
   B2; UK, Germany EP 2 002 007 B1) license holder Targeting Systems, El Cajon, CA.
- UCSD Invention disclosure (Dec., 2010). Cvario: A new pliable biophotonic material with low degradation in seawater. UCSD docket# (in process). (Deheyn DD)
- UC Berkeley γ-PFD filaments to template organic semi-conductors (Monash University, Australia)
- UC Berkeley γ-PFD filaments for magnetically driven protein assembly (Rice University)
- Northwestern Invention disclosure filed on Functionalization of Anisotropic Nanostructures - NU 2010-094
- Northwestern Invention disclosure filed on Short-Duplex Probes for Enhanced Target Nucleic Acid Hybridization - NU 29147
- Northwestern Nanoflare technology licensed to Aurasense.